

IC20 Rec'd PCT/PTO 2 6 SEP 2005

DIARYLMETHYLIDENE PIPERIDINE DERIVATIVES, PREPARATIONS

THEREOF AND USES THEREOF

FIELD OF THE INVENTION

5           The present invention is directed to novel compounds, to a process for their preparation, their use and pharmaceutical compositions comprising the novel compounds. The novel compounds are useful in therapy, and in particular for the treatment of pain, anxiety and functional gastrointestinal disorders.

10   BACKGROUND OF THE INVENTION

          The  $\delta$  receptor has been identified as having a role in many bodily functions such as circulatory and pain systems. Ligands for the  $\delta$  receptor may therefore find potential use as analgesics, and/or as antihypertensive agents. Ligands for the  $\delta$  receptor have also been shown to possess immunomodulatory activities.

15           The identification of at least three different populations of opioid receptors ( $\mu$ ,  $\delta$  and  $\kappa$ ) is now well established and all three are apparent in both central and peripheral nervous systems of many species including man. Analgesia has been observed in various animal models when one or more of these receptors has been activated.

20           With few exceptions, currently available selective opioid  $\delta$  ligands are peptidic in nature and are unsuitable for administration by systemic routes. One example of a non-peptidic  $\delta$ -agonist is SNC80 (Bilsky E.J. et al., Journal of Pharmacology and Experimental Therapeutics, 273(1), pp. 359-366 (1995)).

          Many  $\delta$  agonist compounds that have been identified in the prior art have  
25   many disadvantages in that they suffer from poor pharmacokinetics and are not analgesic when administered by systemic routes. Also, it has been documented that many of these  $\delta$  agonist compounds show significant convulsive effects when administered systemically.

          U.S. Patent No. 6,187,792 to Delorme et al. describes some  $\delta$ -agonists.

30           However, there is still a need for improved  $\delta$ -agonists.

**DESCRIPTION OF THE INVENTION**

Unless specified otherwise within this specification, the nomenclature used in this specification generally follows the examples and rules stated in *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*, Pergamon Press, Oxford, 1979,  
5 which is incorporated by references herein for its exemplary chemical structure names and rules on naming chemical structures.

The term " $C_{m-n}$ " or " $C_{m-n}$  group" used alone or as a prefix, refers to any group having m to n carbon atoms.

The term "hydrocarbon" used alone or as a suffix or prefix, refers to any  
10 structure comprising only carbon and hydrogen atoms up to 14 carbon atoms.

The term "hydrocarbon radical" or "hydrocarbyl" used alone or as a suffix or prefix, refers to any structure as a result of removing one or more hydrogens from a hydrocarbon.

The term "alkyl" used alone or as a suffix or prefix, refers to monovalent  
15 straight or branched chain hydrocarbon radicals comprising 1 to about 12 carbon atoms.

The term "alkylene" used alone or as suffix or prefix, refers to divalent straight or branched chain hydrocarbon radicals comprising 1 to about 12 carbon atoms, which serves to links two structures together.

The term "alkenyl" used alone or as suffix or prefix, refers to a monovalent  
20 straight or branched chain hydrocarbon radical having at least one carbon-carbon double bond and comprising at least 2 up to about 12 carbon atoms.

The term "alkynyl" used alone or as suffix or prefix, refers to a monovalent straight or branched chain hydrocarbon radical having at least one carbon-carbon  
25 triple bond and comprising at least 2 up to about 12 carbon atoms.

The term "cycloalkyl," used alone or as suffix or prefix, refers to a monovalent ring-containing hydrocarbon radical comprising at least 3 up to about 12 carbon atoms.

The term "cycloalkenyl" used alone or as suffix or prefix, refers to a  
30 monovalent ring-containing hydrocarbon radical having at least one carbon-carbon double bond and comprising at least 3 up to about 12 carbon atoms.

The term "cycloalkynyl" used alone or as suffix or prefix, refers to a monovalent ring-containing hydrocarbon radical having at least one carbon-carbon triple bond and comprising about 7 up to about 12 carbon atoms.

5 The term "aryl" used alone or as suffix or prefix, refers to a monovalent hydrocarbon radical having one or more polyunsaturated carbon rings having aromatic character, (*e.g.*,  $4n + 2$  delocalized electrons) and comprising 5 up to about 14 carbon atoms.

10 The term "arylene" used alone or as suffix or prefix, refers to a divalent hydrocarbon radical having one or more polyunsaturated carbon rings having aromatic character, (*e.g.*,  $4n + 2$  delocalized electrons) and comprising 5 up to about 14 carbon atoms, which serves to link two structures together.

15 The term "heterocycle" used alone or as a suffix or prefix, refers to a ring-containing structure or molecule having one or more multivalent heteroatoms, independently selected from N, O, P and S, as a part of the ring structure and including at least 3 and up to about 20 atoms in the ring(s). Heterocycle may be saturated or unsaturated, containing one or more double bonds, and heterocycle may contain more than one ring. When a heterocycle contains more than one ring, the rings may be fused or unfused. Fused rings generally refer to at least two rings share two atoms therebetween. Heterocycle may have aromatic character or may not have aromatic character.

20 The term "heteroaromatic" used alone or as a suffix or prefix, refers to a ring-containing structure or molecule having one or more multivalent heteroatoms, independently selected from N, O, P and S, as a part of the ring structure and including at least 3 and up to about 20 atoms in the ring(s), wherein the ring-containing structure or molecule has an aromatic character (*e.g.*,  $4n + 2$  delocalized electrons).

The term "heterocyclic group," "heterocyclic moiety," "heterocyclic," or "heterocyclo" used alone or as a suffix or prefix, refers to a radical derived from a heterocycle by removing one or more hydrogens therefrom.

30 The term "heterocyclyl" used alone or as a suffix or prefix, refers a monovalent radical derived from a heterocycle by removing one hydrogen therefrom.

The term "heterocyclylene" used alone or as a suffix or prefix, refers to a divalent radical derived from a heterocycle by removing two hydrogens therefrom, which serves to links two structures together.

5 The term "heteroaryl" used alone or as a suffix or prefix, refers to a heterocyclyl having aromatic character.

The term "heterocylcoalkyl" used alone or as a suffix or prefix, refers to a heterocyclyl that does not have aromatic character.

The term "heteroarylene" used alone or as a suffix or prefix, refers to a heterocyclylene having aromatic character.

10 The term "heterocycloalkylene" used alone or as a suffix or prefix, refers to a heterocyclylene that does not have aromatic character.

The term "six-membered" used as prefix refers to a group having a ring that contains six ring atoms.

15 The term "five-membered" used as prefix refers to a group having a ring that contains five ring atoms.

A five-membered ring heteroaryl is a heteroaryl with a ring having five ring atoms wherein 1, 2 or 3 ring atoms are independently selected from N, O and S.

20 Exemplary five-membered ring heteroaryls are thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, isoxazolyl, 1,2,3-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-triazolyl, 1,2,4-thiadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-triazolyl, 1,3,4-thiadiazolyl, and 1,3,4- oxadiazolyl.

A six-membered ring heteroaryl is a heteroaryl with a ring having six ring atoms wherein 1, 2 or 3 ring atoms are independently selected from N, O and S.

25 Exemplary six-membered ring heteroaryls are pyridyl, pyrazinyl, pyrimidinyl, triazinyl and pyridazinyl.

The term "substituted" used as a prefix refers to a structure, molecule or group, wherein one or more hydrogens are replaced with one or more C<sub>1</sub>-hydrocarbon groups, or one or more chemical groups containing one or more heteroatoms selected from N, O, S, F, Cl, Br, I, and P. Exemplary chemical groups  
30 containing one or more heteroatoms include -NO<sub>2</sub>, -OR, -Cl, -Br, -I, -F, -CF<sub>3</sub>, -C(=O)R, -C(=O)OH, -NH<sub>2</sub>, -SH, -NHR, -NR<sub>2</sub>, -SR, -SO<sub>3</sub>H, -SO<sub>2</sub>R, -S(=O)R, -CN, -OH, -C(=O)OR, -C(=O)NR<sub>2</sub>, -NRC(=O)R, oxo (=O), imino (=NR), thio (=S), and

oximino (=N-OR), wherein each "R" is a C<sub>1-6</sub>hydrocarbyl. For example, substituted phenyl may refer to nitrophenyl, methoxyphenyl, chlorophenyl, aminophenyl, etc., wherein the nitro, methoxy, chloro, and amino groups may replace any suitable hydrogen on the phenyl ring.

5       The term "substituted" used as a suffix of a first structure, molecule or group, followed by one or more names of chemical groups refers to a second structure, molecule or group, which is a result of replacing one or more hydrogens of the first structure, molecule or group with the one or more named chemical groups. For example, a "phenyl substituted by nitro" refers to nitrophenyl.

10       The term "optionally substituted" refers to both groups, structures, or molecules that are substituted and those that are not substituted.

Heterocycle includes, for example, monocyclic heterocycles such as: aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, pyrroline, imidazolidine, pyrazolidine, pyrazoline, dioxolane, sulfolane 2,3-dihydrofuran, 2,5-  
15   dihydrofuran tetrahydrofuran, thiophane, piperidine, 1,2,3,6-tetrahydro-pyridine, piperazine, morpholine, thiomorpholine, pyran, thiopyran, 2,3-dihydropyran, tetrahydropyran, 1,4-dihydropyridine, 1,4-dioxane, 1,3-dioxane, dioxane, homopiperidine, 2,3,4,7-tetrahydro-1*H*-azepine homopiperazine, 1,3-dioxepane, 4,7-dihydro-1,3-dioxepin, and hexamethylene oxide.

20       In addition, heterocycle includes aromatic heterocycles, for example, pyridine, pyrazine, pyrimidine, pyridazine, thiophene, furan, furazan, pyrrole, imidazole, thiazole, oxazole, pyrazole, isothiazole, isoxazole, 1,2,3-triazole, tetrazole, 1,2,3-thiadiazole, 1,2,3-oxadiazole, 1,2,4-triazole, 1,2,4-thiadiazole, 1,2,4-oxadiazole, 1,3,4-triazole, 1,3,4-thiadiazole, and 1,3,4- oxadiazole.

25       Additionally, heterocycle encompass polycyclic heterocycles, for example, indole, indoline, isoindoline, quinoline, tetrahydroquinoline, isoquinoline, tetrahydroisoquinoline, 1,4-benzodioxan, coumarin, dihydrocoumarin, benzofuran, 2,3-dihydrobenzofuran, isobenzofuran, chromene, chroman, isochroman, xanthene, phenoxathiin, thianthrene, indolizine, isoindole, indazole, purine, phthalazine,  
30   naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, phenanthridine, perimidine, phenanthroline, phenazine, phenothiazine, phenoxazine, 1,2-benzisoxazole, benzothiophene, benzoxazole, benzthiazole, benzimidazole,

benztriazole, thioxanthine, carbazole, carboline, acridine, pyrrolizidine, and quinolizidine.

In addition to the polycyclic heterocycles described above, heterocycle includes polycyclic heterocycles wherein the ring fusion between two or more rings includes more than one bond common to both rings and more than two atoms common to both rings. Examples of such bridged heterocycles include quinuclidine, diazabicyclo[2.2.1]heptane and 7-oxabicyclo[2.2.1]heptane.

Heterocyclyl includes, for example, monocyclic heterocyclyls, such as: aziridinyl, oxiranyl, thiiranyl, azetidiny, oxetanyl, thietanyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, pyrazolidinyl, pyrazolinyl, dioxolanyl, sulfolanyl, 2,3-dihydrofuranyl, 2,5-dihydrofuranyl, tetrahydrofuranyl, thiophanyl, piperidinyl, 1,2,3,6-tetrahydro-pyridinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyranyl, thiopyranyl, 2,3-dihdropyranyl, tetrahydropyranyl, 1,4-dihdropyridinyl, 1,4-dioxanyl, 1,3-dioxanyl, dioxanyl, homopiperidinyl, 2,3,4,7-tetrahydro-1*H*-azepinyl, homopiperazinyl, 1,3-dioxepanyl, 4,7-dihydro-1,3-dioxepinyl, and hexamethylene oxidyl.

In addition, heterocyclyl includes aromatic heterocyclyls or heteroaryl, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, thienyl, furyl, furazanyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, isoxazolyl, 1,2,3-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-triazolyl, 1,2,4-thiadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-triazolyl, 1,3,4-thiadiazolyl, and 1,3,4-oxadiazolyl.

Additionally, heterocyclyl encompasses polycyclic heterocyclyls (including both aromatic or non-aromatic), for example, indolyl, indolinyl, isoindolinyl, quinolinyl, tetrahydroquinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, 1,4-benzodioxanyl, coumarinyl, dihydrocoumarinyl, benzofuranyl, 2,3-dihydrobenzofuranyl, isobenzofuranyl, chromenyl, chromanyl, isochromanyl, xanthenyl, phenoxathiinyl, thianthrenyl, indolizinyl, isoindolyl, indazolyl, purinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, phenanthridinyl, perimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxazinyl, 1,2-benzisoxazolyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benzimidazolyl, benztriazolyl, thioxanthinyl, carbazolyl, carbolinyl, acridinyl, pyrrolizidinyl, and quinolizidinyl.

In addition to the polycyclic heterocyclyls described above, heterocyclyl includes polycyclic heterocyclyls wherein the ring fusion between two or more rings includes more than one bond common to both rings and more than two atoms common to both rings. Examples of such bridged heterocycles include quinuclidinyl,  
 5 diazabicyclo[2.2.1]heptyl; and 7-oxabicyclo[2.2.1]heptyl.

The term "alkoxy" used alone or as a suffix or prefix, refers to radicals of the general formula  $-O-R$ , wherein R is selected from a hydrocarbon radical. Exemplary alkoxy includes methoxy, ethoxy, propoxy, isopropoxy, butoxy, t-butoxy, isobutoxy, cyclopropylmethoxy, allyloxy, and propargyloxy.

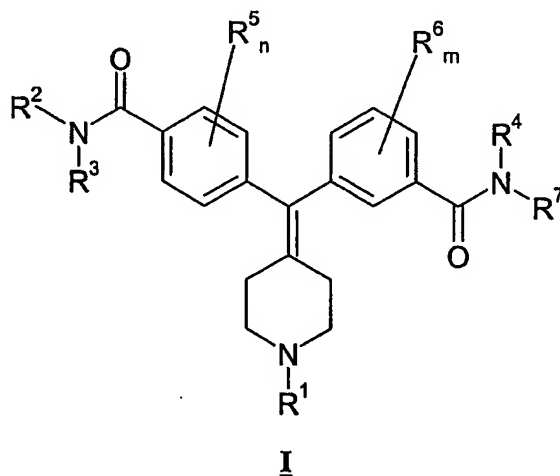
10 The term "amine" or "amino" used alone or as a suffix or prefix, refers to radicals of the general formula  $-NRR'$ , wherein R and R' are independently selected from hydrogen or a hydrocarbon radical.

Halogen includes fluorine, chlorine, bromine and iodine.

"Halogenated," used as a prefix of a group, means one or more hydrogens on  
 15 the group is replaced with one or more halogens.

"RT" or "rt" means room temperature.

In one embodiment, the invention provides a compound of formula I, a pharmaceutically acceptable salt thereof, diastereomers thereof, enantiomers thereof, and mixtures thereof:



wherein

R¹ is hydrogen, C<sub>1-6</sub>alkyl-O-C(=O)-, C<sub>1-6</sub>alkyl, substituted C<sub>1-6</sub>alkyl,  
 25 C<sub>3-6</sub>cycloalkyl, and substituted C<sub>3-6</sub>cycloalkyl, optionally substituted aryl, optionally

substituted heteroaryl, optionally substituted arylalkyl or optionally substituted heteroarylalkyl;

n is 0, 1 or 2; m is 0, 1, or 2;

$R^2$ ,  $R^3$  and  $R^4$  are, independently, selected from hydrogen,  $C_{1-6}$ alkyl, substituted  $C_{1-6}$ alkyl,  $C_{3-6}$ cycloalkyl, and substituted  $C_{3-6}$ cycloalkyl;

$R^5$  and  $R^6$  are, independently, selected from -R, -NO<sub>2</sub>, -OR, -Cl, -Br, -I, -F, -CF<sub>3</sub>, -C(=O)R, -C(=O)OH, -NH<sub>2</sub>, -SH, -NHR, -NR<sub>2</sub>, -SR, -SO<sub>3</sub>H, -SO<sub>2</sub>R, -S(=O)R, -CN, -OH, -C(=O)OR, -C(=O)NR<sub>2</sub>, -NRC(=O)R, and -NRC(=O)-OR, wherein R is, independently, a hydrogen or  $C_{1-6}$ alkyl; and

$R^7$  is selected from  $C_{1-6}$ alkyl, substituted  $C_{1-6}$ alkyl,  $C_{3-6}$ cycloalkyl, and substituted  $C_{3-6}$ cycloalkyl, optionally substituted  $C_{6-10}$ aryl, optionally substituted  $C_{3-9}$ heteroaryl, optionally substituted  $C_{6-10}$ aryl- $C_{1-6}$ alkyl, and optionally substituted  $C_{3-9}$ heteroaryl- $C_{1-6}$ alkyl; or  $R^4$  and  $R^7$  together with nitrogen connected thereto form a portion of a  $C_{3-6}$ heterocycle ring.

Particularly, the compounds of the present invention are those of formula I, wherein  $R^1$  is hydrogen,  $C_{1-6}$ alkyl-O-C(=O)-,  $C_{1-6}$ alkyl, substituted  $C_{1-6}$ alkyl,  $C_{3-6}$ cycloalkyl, and substituted  $C_{3-6}$ cycloalkyl;

$R^2$  and  $R^3$  are, independently,  $C_{1-3}$ alkyl or halogenated  $C_{1-3}$ alkyl;

$R^4$  is hydrogen,  $C_{1-6}$ alkyl, substituted  $C_{1-6}$ alkyl,  $C_{3-6}$ cycloalkyl, or substituted  $C_{3-6}$ cycloalkyl;

$R^7$  is selected from optionally substituted  $C_{6-10}$ aryl, optionally substituted  $C_{3-9}$ heteroaryl, optionally substituted  $C_{6-10}$ aryl- $C_{1-6}$ alkyl, and optionally substituted  $C_{3-9}$ heteroaryl- $C_{1-6}$ alkyl; and

n and m are 0.

More particularly, the compounds of the present invention are those of formula I, wherein  $R^1$  is selected from hydrogen,  $C_{1-6}$ alkyl-O-C(=O)-;

$R^2$  and  $R^3$  are ethyl;

$R^4$  is hydrogen;

$R^7$  is  $C_{6-10}$ aryl or  $C_{6-10}$ aryl- $C_{1-3}$ alkyl; and

n and m are 0.

Most particularly, the compounds of the present invention are those of formula I, wherein



$R^1$  is hydrogen;

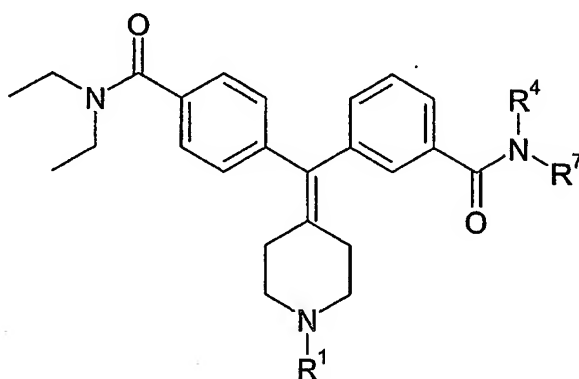
$R^2$  and  $R^3$  are ethyl;

$R^4$  is hydrogen;

$R^7$  is phenyl, benzyl or phenethyl; and

5 n and m are 0.

In another embodiment, the invention provides a compound of formula IA, a pharmaceutically acceptable salt thereof, diastereomers thereof, enantiomers thereof, and mixtures thereof:



IA

10

wherein

$R^1$  is selected from hydrogen, and  $C_{1-6}$ alkyl-O-C(=O)-;

$R^4$  is selected from hydrogen,  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, and  $C_{3-6}$ cycloalkyl, wherein said  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, and  $C_{3-6}$ cycloalkyl are  
 15 optionally substituted with one or more groups selected from -R, -NO<sub>2</sub>, -OR, -Cl, -Br, -I, -F, -CF<sub>3</sub>, -C(=O)R, -C(=O)OH, -NH<sub>2</sub>, -SH, -NHR, -NR<sub>2</sub>, -SR, -SO<sub>3</sub>H, -SO<sub>2</sub>R, -S(=O)R, -CN, -OH, -C(=O)OR, -C(=O)NR<sub>2</sub>, -NRC(=O)R, and -NRC(=O)-OR, wherein R is, independently, a hydrogen or  $C_{1-6}$ alkyl;

$R^7$  is selected from  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl,  $C_{3-6}$ cycloalkyl,  $C_{3-6}$ cycloalkyl- $C_{1-3}$ alkyl,  $C_{6-10}$ aryl,  $C_{6-10}$ aryl- $C_{1-3}$ alkyl,  $C_{3-6}$ heteroaryl, and  $C_{3-6}$ heteroaryl- $C_{1-3}$ alkyl, wherein said  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl,  $C_{3-6}$ cycloalkyl,  $C_{3-6}$ cycloalkyl- $C_{1-3}$ alkyl,  $C_{6-10}$ aryl,  $C_{6-10}$ aryl- $C_{1-3}$ alkyl,  $C_{3-6}$ heteroaryl, and  $C_{3-6}$ heteroaryl- $C_{1-3}$ alkyl are optionally substituted with one or more groups selected from  
 20 -R, -NO<sub>2</sub>, -OR, -Cl, -Br, -I, -F, -CF<sub>3</sub>, -C(=O)R, -C(=O)OH, -NH<sub>2</sub>, -SH, -NHR, -NR<sub>2</sub>, -SR, -SO<sub>3</sub>H, -SO<sub>2</sub>R, -S(=O)R, -CN, -OH, -C(=O)OR, -C(=O)NR<sub>2</sub>, -NRC(=O)R, and  
 25

-NRC(=O)-OR, wherein R is, independently, a hydrogen or C<sub>1-6</sub>alkyl; or R<sup>4</sup> and R<sup>7</sup> together with nitrogen connected thereto form a portion of a C<sub>3-6</sub>heterocycle ring.

A further embodiment of the invention is a compound of formula IA, wherein R<sup>1</sup> is hydrogen;

5 R<sup>4</sup> is selected from hydrogen and C<sub>1-6</sub>alkyl; and

R<sup>7</sup> is selected from C<sub>3-6</sub>alkyl, C<sub>3-6</sub>cycloalkyl, C<sub>3-6</sub>cycloalkyl-C<sub>1-3</sub>alkyl, phenyl, phenyl-C<sub>1-3</sub>alkyl, and C<sub>3-6</sub>heteroaryl, wherein said R<sup>7</sup> is further optionally substituted with one or more groups selected from C<sub>1-6</sub>alkyl, halogenated C<sub>1-6</sub>alkyl, -NO<sub>2</sub>, -CF<sub>3</sub>, C<sub>1-6</sub>alkoxy, chloro, fluoro, bromo, and iodo.

10 An even further embodiment of the invention is a compound of formula IA, wherein R<sup>1</sup> is hydrogen;

R<sup>4</sup> is selected from hydrogen and methyl; and

R<sup>7</sup> is selected from C<sub>4-6</sub>alkyl, phenyl, benzyl, 2-phenylethyl, 1-phenylethyl, cyclopentyl, thiazolyl, pyridinyl and cyclohexyl, wherein R<sup>7</sup> is further optionally  
15 substituted with one or more groups selected from methyl, methoxy, chloro, and fluoro.

Another embodiment of the invention is a compound of formula IA, wherein R<sup>1</sup> is hydrogen; and

R<sup>4</sup> and R<sup>7</sup> are directly linked to form a divalent C<sub>3-6</sub>alkylene, wherein said C<sub>3-6</sub>alkylene is optionally substituted with one or more groups selected from methyl,  
20 methoxy, chloro, and fluoro.

An even further embodiment of the invention is a compound of formula IA, wherein R<sup>1</sup> is hydrogen; and

R<sup>4</sup> and R<sup>7</sup> are directly linked to form 1,5-pentylene or 1,4-butylene.

25 It will be understood that when compounds of the present invention contain one or more chiral centers, the compounds of the invention may exist in, and be isolated as, enantiomeric or diastereomeric forms, or as a racemic mixture. The present invention includes any possible enantiomers, diastereomers, racemates or mixtures thereof, of a compound of Formula I or IA. The optically active forms of the  
30 compound of the invention may be prepared, for example, by chiral chromatographic separation of a racemate, by synthesis from optically active starting materials or by asymmetric synthesis based on the procedures described thereafter.

It will also be appreciated that certain compounds of the present invention may exist as geometrical isomers, for example E and Z isomers of alkenes. The present invention includes any geometrical isomer of a compound of Formula I or IA. It will further be understood that the present invention encompasses tautomers of the  
5 compounds of the Formula I or IA.

It will also be understood that certain compounds of the present invention may exist in solvated, for example hydrated, as well as unsolvated forms. It will further be understood that the present invention encompasses all such solvated forms of the compounds of the Formula I or IA.

10 Within the scope of the invention are also salts of the compounds of the Formula I or IA. Generally, pharmaceutically acceptable salts of compounds of the present invention may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound, for example an alkyl amine with a suitable acid, for example, HCl or acetic acid, to afford a physiologically  
15 acceptable anion. It may also be possible to make a corresponding alkali metal (such as sodium, potassium, or lithium) or an alkaline earth metal (such as a calcium) salt by treating a compound of the present invention having a suitably acidic proton, such as a carboxylic acid or a phenol with one equivalent of an alkali metal or alkaline  
20 earth metal hydroxide or alkoxide (such as the ethoxide or methoxide), or a suitably basic organic amine (such as choline or meglumine) in an aqueous medium, followed by conventional purification techniques.

In one embodiment, the compound of Formula I or IA above may be converted to a pharmaceutically acceptable salt or solvate thereof, particularly, an acid addition salt such as a hydrochloride, hydrobromide, phosphate, acetate,  
25 fumarate, maleate, tartrate, citrate, methanesulphonate or *p*-toluenesulphonate.

The novel compounds of the present invention are useful in therapy, especially for the treatment of various pain conditions such as chronic pain, neuropathic pain, acute pain, cancer pain, pain caused by rheumatoid arthritis, migraine, visceral pain etc. This list should however not be interpreted as exhaustive.

30 Compounds of the invention are useful as immunomodulators, especially for autoimmune diseases, such as arthritis, for skin grafts, organ transplants and similar

surgical needs, for collagen diseases, various allergies, for use as anti-tumour agents and anti viral agents.

Compounds of the invention are useful in disease states where degeneration or dysfunction of opioid receptors is present or implicated in that paradigm. This may  
5 involve the use of isotopically labelled versions of the compounds of the invention in diagnostic techniques and imaging applications such as positron emission tomography (PET).

Compounds of the invention are useful for the treatment of diarrhoea, depression, anxiety and stress-related disorders such as post-traumatic stress  
10 disorders, panic disorder, generalized anxiety disorder, social phobia, and obsessive compulsive disorder, urinary incontinence, premature ejaculation, various mental illnesses, cough, lung oedema, various gastro-intestinal disorders, e.g. constipation, functional gastrointestinal disorders such as Irritable Bowel Syndrome and Functional Dyspepsia, Parkinson's disease and other motor disorders, traumatic brain injury,  
15 stroke, cardioprotection following myocardial infarction, spinal injury and drug addiction, including the treatment of alcohol, nicotine, opioid and other drug abuse and for disorders of the sympathetic nervous system for example hypertension.

Compounds of the invention are useful as an analgesic agent for use during general anaesthesia and monitored anaesthesia care. Combinations of agents with  
20 different properties are often used to achieve a balance of effects needed to maintain the anaesthetic state (e.g. amnesia, analgesia, muscle relaxation and sedation). Included in this combination are inhaled anaesthetics, hypnotics, anxiolytics, neuromuscular blockers and opioids.

Also within the scope of the invention is the use of any of the compounds  
25 according to the Formula I or IA above, for the manufacture of a medicament for the treatment of any of the conditions discussed above.

A further aspect of the invention is a method for the treatment of a subject suffering from any of the conditions discussed above, whereby an effective amount of a compound according to the Formula I or IA above, is administered to a patient in  
30 need of such treatment.

Thus, the invention provides a compound of Formula I or IA, or pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined for use in therapy.

In a further aspect, the present invention provides the use of a compound of  
5 Formula I or IA, or a pharmaceutically acceptable salt or solvate thereof, as hereinbefore defined in the manufacture of a medicament for use in therapy.

In the context of the present specification, the term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The term "therapeutic" and "therapeutically" should be construed accordingly. The term  
10 "therapy" within the context of the present invention further encompasses to administer an effective amount of a compound of the present invention, to mitigate either a pre-existing disease state, acute or chronic, or a recurring condition. This definition also encompasses prophylactic therapies for prevention of recurring conditions and continued therapy for chronic disorders.

15 The compounds of the present invention are useful in therapy, especially for the therapy of various pain conditions including, but not limited to: chronic pain, neuropathic pain, acute pain, back pain, cancer pain, and visceral pain.

In use for therapy in a warm-blooded animal such as a human, the compound of the invention may be administered in the form of a conventional pharmaceutical  
20 composition by any route including orally, intramuscularly, subcutaneously, topically, intranasally, intraperitoneally, intrathoracically, intravenously, epidurally, intrathecally, intracerebroventricularly and by injection into the joints.

In one embodiment of the invention, the route of administration may be orally, intravenously or intramuscularly.

25 The dosage will depend on the route of administration, the severity of the disease, age and weight of the patient and other factors normally considered by the attending physician, when determining the individual regimen and dosage level at the most appropriate for a particular patient.

For preparing pharmaceutical compositions from the compounds of this  
30 invention, inert, pharmaceutically acceptable carriers can be either solid and liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, and suppositories.

A solid carrier can be one or more substances, which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or table disintegrating agents; it can also be an encapsulating material.

5 In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided compound of the invention, or the active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

10 For preparing suppository compositions, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized moulds and allowed to cool and solidify.

Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

15 The term composition is also intended to include the formulation of the active component with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier which is thus in association with it. Similarly, cachets are included.

20 Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form compositions include solutions, suspensions, and emulsions. For example, sterile water or water propylene glycol solutions of the active compounds may be liquid preparations suitable for parenteral administration. Liquid compositions can also be formulated in solution in aqueous polyethylene glycol solution.

25 Aqueous solutions for oral administration can be prepared by dissolving the active component in water and adding suitable colorants, flavoring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other suspending agents known to the pharmaceutical formulation art.

Depending on the mode of administration, the pharmaceutical composition will preferably include from 0.05% to 99%w (per cent by weight), more preferably from 0.10 to 50%w, of the compound of the invention, all percentages by weight being based on total composition.

5 A therapeutically effective amount for the practice of the present invention may be determined, by the use of known criteria including the age, weight and response of the individual patient, and interpreted within the context of the disease which is being treated or which is being prevented, by one of ordinary skills in the art.

Within the scope of the invention is the use of any compound of Formula I or  
10 IA as defined above for the manufacture of a medicament.

Also within the scope of the invention is the use of any compound of Formula I or IA for the manufacture of a medicament for the therapy of pain.

Additionally provided is the use of any compound according to Formula I or IA for the manufacture of a medicament for the therapy of various pain conditions  
15 including, but not limited to: chronic pain, neuropathic pain, acute pain, back pain, cancer pain, and visceral pain.

A further aspect of the invention is a method for therapy of a subject suffering from any of the conditions discussed above, whereby an effective amount of a compound according to the Formula I or IA above, is administered to a patient in need  
20 of such therapy.

Additionally, there is provided a pharmaceutical composition comprising a compound of Formula I or IA, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

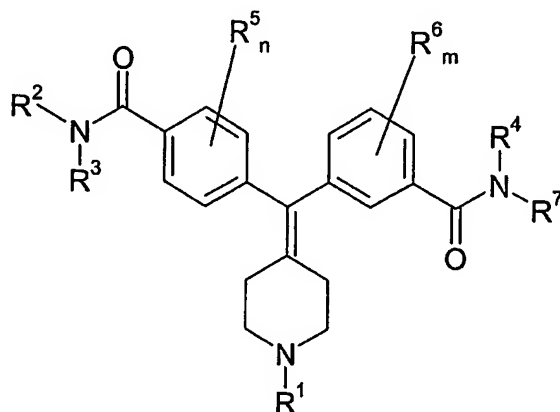
Particularly, there is provided a pharmaceutical composition comprising a  
25 compound of Formula I or IA, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier for therapy, more particularly for therapy of pain.

Further, there is provided a pharmaceutical composition comprising a compound of Formula I or IA, or a pharmaceutically acceptable salt thereof, in  
30 association with a pharmaceutically acceptable carrier use in any of the conditions discussed above.

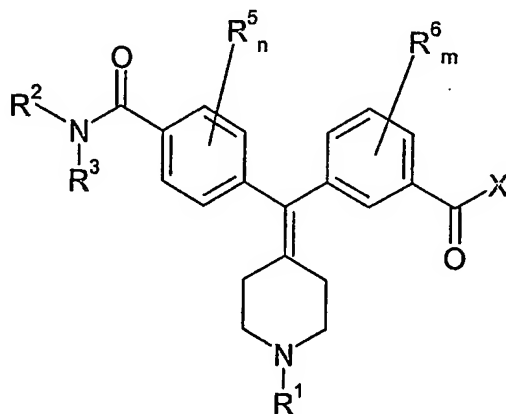
16

In a further aspect, the present invention provides a method of preparing a compound of Formula I or IA.

In one embodiment, the invention provides a process for preparing a compound of formula I, comprising of the step of

I

reacting a compound of formula II with  $\text{HNR}^4\text{R}^7$ :

II

wherein

$\text{R}^1$  is hydrogen,  $\text{C}_{1-6}$ alkyl- $\text{O}-\text{C}(=\text{O})-$ ,  $\text{C}_{1-6}$ alkyl, substituted  $\text{C}_{1-6}$ alkyl,  $\text{C}_{3-6}$ cycloalkyl, and substituted  $\text{C}_{3-6}$ cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted arylalkyl or optionally substituted heteroarylalkyl;

$n$  is 0, 1 or 2;  $m$  is 0, 1, or 2;



X is selected from -OH, -OR<sup>8</sup>, -O-C(=O)-R<sup>8</sup>, -Cl, -Br and -I, wherein R<sup>8</sup> is C<sub>1-6</sub>alkyl;

R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are, independently, selected from hydrogen, C<sub>1-6</sub>alkyl, substituted C<sub>1-6</sub>alkyl, C<sub>3-6</sub>cycloalkyl, and substituted C<sub>3-6</sub>cycloalkyl;

5 R<sup>5</sup> and R<sup>6</sup> are, independently, selected from -R, -NO<sub>2</sub>, -OR, -Cl, -Br, -I, -F, -CF<sub>3</sub>, -C(=O)R, -C(=O)OH, -NH<sub>2</sub>, -SH, -NHR, -NR<sub>2</sub>, -SR, -SO<sub>3</sub>H, -SO<sub>2</sub>R, -S(=O)R, -CN, -OH, -C(=O)OR, -C(=O)NR<sub>2</sub>, -NRC(=O)R, and -NRC(=O)-OR, wherein R is, independently, a hydrogen or C<sub>1-6</sub>alkyl; and

10 R<sup>7</sup> is C<sub>1-6</sub>alkyl, substituted C<sub>1-6</sub>alkyl, C<sub>3-6</sub>cycloalkyl, and substituted C<sub>3-6</sub>cycloalkyl, optionally substituted C<sub>6-10</sub>aryl, optionally substituted C<sub>3-9</sub>heteroaryl, optionally substituted C<sub>6-10</sub>aryl-C<sub>1-6</sub>alkyl, and optionally substituted C<sub>3-9</sub>heteroaryl-C<sub>1-6</sub>alkyl; or R<sup>4</sup> and R<sup>7</sup> together with nitrogen connected thereto form a portion of a C<sub>3-6</sub>heterocycle ring.

15 In a particular embodiment, the invention provides a process for preparing a compound of formula I as described above, wherein X is -OH;

R<sup>1</sup> is C<sub>1-6</sub>alkyl-O-C(=O)-;

R<sup>2</sup> and R<sup>3</sup> are ethyl;

R<sup>4</sup> is hydrogen or methyl;

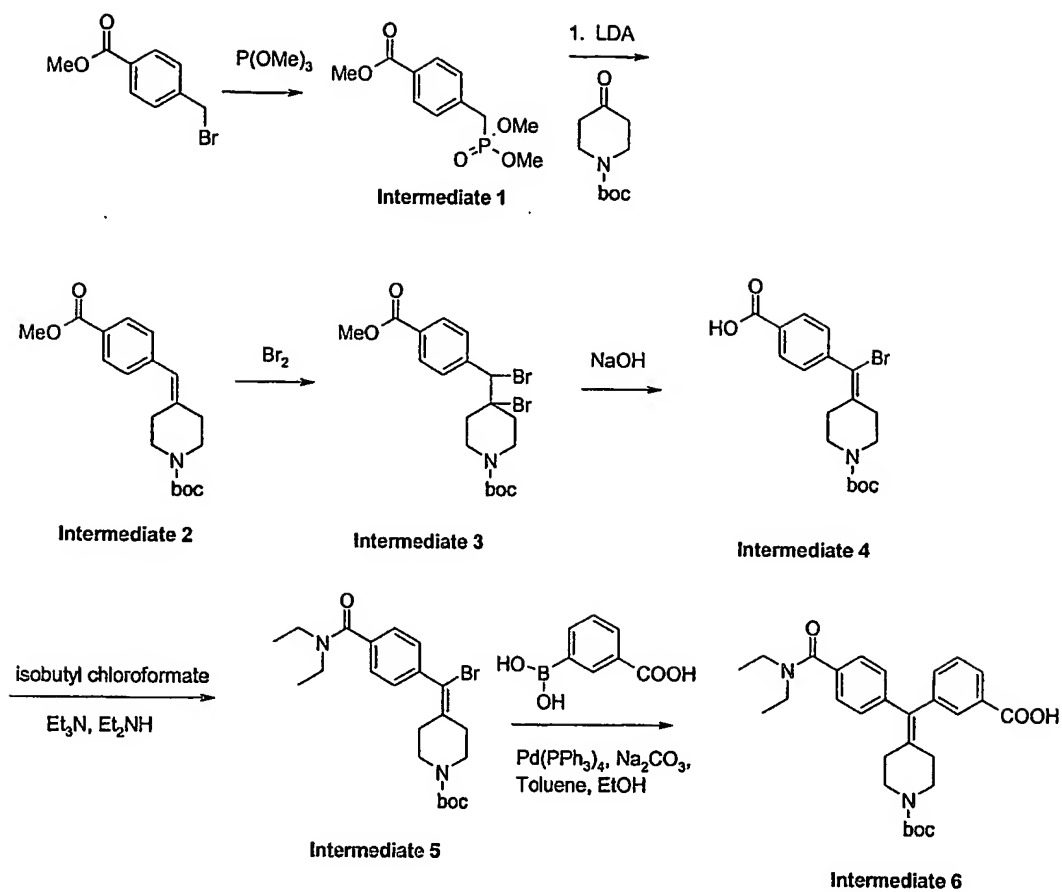
20 R<sup>7</sup> is phenyl, benzyl, phenethyl, cyclopentyl, cyclohexyl, cyclohexylmethyl, 2-chlorobenzyl, 2-fluorobenzyl, 1-(4-methylphenyl)ethyl, 4-methyl-1,3-thiazol-2-yl, 2,6-dimethylpyridin-3-yl, isobutyl, or 1-ethylpropyl; or R<sup>4</sup> and R<sup>7</sup> together form 1,5-pentylene or 1,4-butylene; and

n and m are 0.

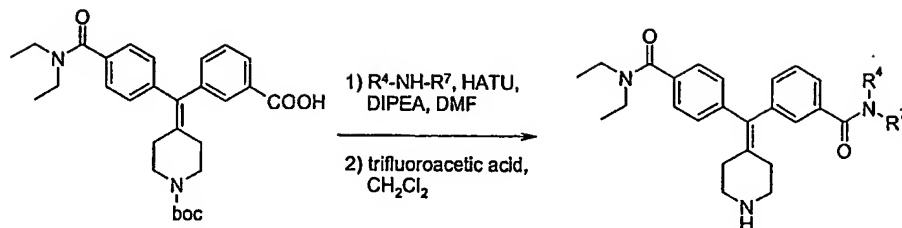
25 More particularly, the compounds of the present invention and intermediates used for the preparation thereof can be prepared according to the synthetic routes as exemplified in Schemes 1 and 2.

18

Scheme 1



Scheme 2



Intermediate 6

Compound 1:  $R^7$ =phenyl,  $R^4$ =H  
 Compound 2:  $R^7$ =benzyl,  $R^4$ =H  
 Compound 3:  $R^7$ =phenylethyl,  $R^4$ =H  
 Compound 4:  $R^7$ =cyclopentyl,  $R^4$ =H  
 Compound 5:  $R^7$ =cyclohexyl,  $R^4$ =H  
 Compound 6:  $R^7$ =cyclohexylmethyl,  $R^4$ =H  
 Compound 7:  $R^7$ =2-chlorobenzyl,  $R^4$ =H  
 Compound 8:  $R^7$ =2-fluorobenzyl,  $R^4$ =H  
 Compound 9:  $R^7$ =1-(4-methylphenyl)ethyl,  $R^4$ =H  
 Compound 10:  $R^7$ =4-methyl-1,3-thiazol-2-yl,  $R^4$ =H  
 Compound 11:  $R^7$ =2,6-dimethylpyridin-3-yl,  $R^4$ =H  
 Compound 12:  $R^7$ =isobutyl,  $R^4$ =H  
 Compound 13:  $R^7$ =1-ethylpropyl,  $R^4$ =H  
 Compound 14:  $R^7$ =phenylethyl,  $R^4$ =methyl  
 Compound 15:  $R^7+R^4$ =-CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>-  
 Compound 16:  $R^7+R^4$ =-CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>-

## BIOLOGICAL EVALUATION

The compounds of the invention are found to be active towards  $\delta$  receptors in warm-blooded animal, e.g., human. Particularly the compounds of the invention are found to be effective  $\delta$  receptor ligands. *In vitro* assays, *infra*, demonstrate these surprising activities, especially with regard to agonists potency and efficacy as demonstrated in the rat brain functional assay and/or the human  $\delta$  receptor functional assay. This feature may be related to *in vivo* activity and may not be linearly correlated with binding affinity. In these *in vitro* assays, a compound is tested for their activity toward  $\delta$  receptors and IC<sub>50</sub> is obtained to determine the selective activity for a particular compound towards  $\delta$  receptors. In the current context, IC<sub>50</sub> generally refers to the concentration of the compound at which 50% displacement of a standard radioactive  $\delta$  receptor ligand has been observed.

The activities of the compound towards  $\kappa$  and  $\mu$  receptors are also measured in a similar assay.

**In vitro model****Cell culture**

Human 293S cells expressing cloned human  $\kappa$ ,  $\delta$  and  $\mu$  receptors and neomycin resistance are grown in suspension at 37°C and 5% CO<sub>2</sub> in shaker flasks  
5 containing calcium-free DMEM 10% FBS, 5% BCS, 0.1% Pluronic F-68, and 600  $\mu$ g/ml geneticin.

Rat brains are weighed and rinsed in ice-cold PBS (containing 2.5mM EDTA, pH 7.4). The brains are homogenized with a polytron for 30 sec (rat) in ice-cold lysis buffer (50mM Tris, pH 7.0, 2.5mM EDTA, with phenylmethylsulfonyl fluoride added  
10 just prior use to 0.5mM from a 0.5M stock in DMSO:ethanol).

**Membrane preparation**

Cells are pelleted and resuspended in lysis buffer (50 mM Tris, pH 7.0, 2.5 mM EDTA, with PMSF added just prior to use to 0.1 mM from a 0.1 M stock in ethanol), incubated on ice for 15 min, then homogenized with a polytron for 30 sec.  
15 The suspension is spun at 1000g (max) for 10 min at 4°C. The supernatant is saved on ice and the pellets resuspended and spun as before. The supernatants from both spins are combined and spun at 46,000 g(max) for 30 min. The pellets are resuspended in cold Tris buffer (50 mM Tris/Cl, pH 7.0) and spun again. The final pellets are resuspended in membrane buffer ( 50 mM Tris, 0.32 M sucrose, pH 7.0). Aliquots (1  
20 ml) in polypropylene tubes are frozen in dry ice/ethanol and stored at -70°C until use. The protein concentrations are determined by a modified Lowry assay with sodium dodecyl sulfate.

**Binding assays**

Membranes are thawed at 37°C, cooled on ice, passed 3 times through a 25-  
25 gauge needle, and diluted into binding buffer (50 mM Tris, 3 mM MgCl<sub>2</sub>, 1 mg/ml BSA (Sigma A-7888), pH 7.4, which is stored at 4°C after filtration through a 0.22  $\mu$ m filter, and to which has been freshly added 5  $\mu$ g/ml aprotinin, 10  $\mu$ M bestatin, 10  $\mu$ M diprotin A, no DTT). Aliquots of 100  $\mu$ l are added to iced 12x75 mm polypropylene tubes containing 100  $\mu$ l of the appropriate radioligand and 100  $\mu$ l of test compound at  
30 various concentrations. Total (TB) and nonspecific (NS) binding are determined in

the absence and presence of 10  $\mu$ M naloxone respectively. The tubes are vortexed and incubated at 25°C for 60-75 min, after which time the contents are rapidly vacuum-filtered and washed with about 12 ml/tube iced wash buffer (50 mM Tris, pH 7.0, 3 mM  $MgCl_2$ ) through GF/B filters (Whatman) presoaked for at least 2h in 0.1% polyethyleneimine. The radioactivity (dpm) retained on the filters is measured with a beta counter after soaking the filters for at least 12h in minivials containing 6-7 ml scintillation fluid. If the assay is set up in 96-place deep well plates, the filtration is over 96-place PEI-soaked unifilters, which are washed with 3 x 1 ml wash buffer, and dried in an oven at 55°C for 2h. The filter plates are counted in a TopCount (Packard) after adding 50  $\mu$ l MS-20 scintillation fluid/well.

### **Functional Assays**

The agonist activity of the compounds is measured by determining the degree to which the compounds receptor complex activates the binding of GTP to G-proteins to which the receptors are coupled. In the GTP binding assay, GTP[ $\gamma$ ]<sup>35</sup>S is combined with test compounds and membranes from HEK-293S cells expressing the cloned human opioid receptors or from homogenised rat and mouse brain. Agonists stimulate GTP[ $\gamma$ ]<sup>35</sup>S binding in these membranes. The EC<sub>50</sub> and E<sub>max</sub> values of compounds are determined from dose-response curves. Right shifts of the dose response curve by the delta antagonist naltrindole are performed to verify that agonist activity is mediated through delta receptors. The E<sub>max</sub> values were determined in relation to the standard  $\delta$  agonist SNC80, i.e., higher than 100% is a compound that have better efficacy than SNC80.

### **Procedure for rat brain GTP**

Rat brain membranes are thawed at 37°C, passed 3 times through a 25-gauge blunt-end needle and diluted in the GTP $\gamma$ S binding (50 mM Hepes, 20 mM NaOH, 100 mM NaCl, 1 mM EDTA, 5 mM  $MgCl_2$ , pH 7.4, Add fresh: 1 mM DTT, 0.1% BSA ). 120 $\mu$ M GDP final is added membranes dilutions. The EC<sub>50</sub> and E<sub>max</sub> of compounds are evaluated from 10-point dose-response curves done in 300 $\mu$ l with the appropriate amount of membrane protein (20 $\mu$ g/well) and 100000-130000 dpm of GTP $\gamma$ <sup>35</sup>S per well (0.11 -0.14nM). The basal and maximal stimulated binding are determined in absence and presence of 3  $\mu$ M SNC-80

**Data analysis**

The specific binding (SB) was calculated as TB-NS, and the SB in the presence of various test compounds was expressed as percentage of control SB. Values of  $IC_{50}$  and Hill coefficient ( $n_H$ ) for ligands in displacing specifically bound radioligand were calculated from logit plots or curve fitting programs such as Ligand, GraphPad Prism, SigmaPlot, or ReceptorFit. Values of  $K_i$  were calculated from the Cheng-Prussoff equation. Mean  $\pm$  S.E.M. values of  $IC_{50}$ ,  $K_i$  and  $n_H$  were reported for ligands tested in at least three displacement curves. Biological activity for some of the compounds of the present invention is indicated in Table 1.

**Table 1**

| Compound.<br># | Human $\delta$ |                |           | Human $\kappa$ | Human $\mu$    |
|----------------|----------------|----------------|-----------|----------------|----------------|
|                | $IC_{50}$ (nM) | $EC_{50}$ (nM) | %EMax (%) | $IC_{50}$ (nM) | $IC_{50}$ (nM) |
| 1-16           | 0.36-9.73      | 22.5-794       | 69-105    | 1600-9000      | 86-8700        |

**Receptor Saturation Experiments**

Radioligand  $K_d$  values are determined by performing the binding assays on cell membranes with the appropriate radioligands at concentrations ranging from 0.2 to 5 times the estimated  $K_d$  (up to 10 times if amounts of radioligand required are feasible). The specific radioligand binding is expressed as pmole/mg membrane protein. Values of  $K_d$  and  $B_{max}$  from individual experiments are obtained from nonlinear fits of specifically bound (B) vs. nM free (F) radioligand from individual according to a one-site model.

**Determination Of Mechano-Allodynia Using Von Frey Testing**

Testing is performed between 08:00 and 16:00h using the method described by Chaplan et al. (1994). Rats are placed in Plexiglas cages on top of a wire mesh bottom which allows access to the paw, and are left to habituate for 10-15 min. The area tested is the mid-plantar left hind paw, avoiding the less sensitive foot pads. The

paw is touched with a series of 8 Von Frey hairs with logarithmically incremental stiffness (0.41, 0.69, 1.20, 2.04, 3.63, 5.50, 8.51, and 15.14 grams; Stoelting, Ill, USA). The von Frey hair is applied from underneath the mesh floor perpendicular to the plantar surface with sufficient force to cause a slight buckling against the paw, and  
 5 held for approximately 6-8 seconds. A positive response is noted if the paw is sharply withdrawn. Flinching immediately upon removal of the hair is also considered a positive response. Ambulation is considered an ambiguous response, and in such cases the stimulus is repeated.

### Testing Protocol

10 The animals are tested on postoperative day 1 for the FCA-treated group. The 50% withdrawal threshold is determined using the up-down method of Dixon (1980). Testing is started with the 2.04 g hair, in the middle of the series. Stimuli are always presented in a consecutive way, whether ascending or descending. In the absence of a paw withdrawal response to the initially selected hair, a stronger stimulus is  
 15 presented; in the event of paw withdrawal, the next weaker stimulus is chosen. Optimal threshold calculation by this method requires 6 responses in the immediate vicinity of the 50% threshold, and counting of these 6 responses begins when the first change in response occurs, e.g. the threshold is first crossed. In cases where thresholds fall outside the range of stimuli, values of 15.14 (normal sensitivity) or  
 20 0.41 (maximally allodynic) are respectively assigned. The resulting pattern of positive and negative responses is tabulated using the convention, X = no withdrawal; O = withdrawal, and the 50% withdrawal threshold is interpolated using the formula:

$$50\% \text{ g threshold} = 10^{(X_f + k\delta)} / 10,000$$

where  $X_f$  = value of the last von Frey hair used (log units);  $k$  = tabular value (from  
 25 Chaplan et al. (1994)) for the pattern of positive / negative responses; and  $\delta$  = mean difference between stimuli (log units). Here  $\delta = 0.224$ .

Von Frey thresholds are converted to percent of maximum possible effect (% MPE), according to Chaplan et al. 1994. The following equation is used to compute % MPE:

30 
$$\% \text{ MPE} = \frac{\text{Drug treated threshold (g)} - \text{allodynia threshold (g)}}{\text{Control threshold (g)} - \text{allodynia threshold (g)}} \times 100$$

### Administration Of Test Substance

Rats are injected (subcutaneously, intraperitoneally, intravenously or orally) with a test substance prior to von Frey testing, the time between administration of test compound and the von Frey test varies depending upon the nature of the test compound.

### Writhing Test

Acetic acid will bring abdominal contractions when administered intraperitoneally in mice. These will then extend their body in a typical pattern. When analgesic drugs are administered, this described movement is less frequently observed and the drug selected as a potential good candidate.

A complete and typical Writhing reflex is considered only when the following elements are present: the animal is not in movement; the lower back is slightly depressed; the plantar aspect of *both* paws is observable. In this assay, compounds of the present invention demonstrate significant inhibition of writhing responses after oral dosing of 1-100  $\mu\text{mol/kg}$ .

#### (i) Solutions preparation

Acetic acid (AcOH): 120  $\mu\text{L}$  of Acetic Acid is added to 19.88 ml of distilled water in order to obtain a final volume of 20 ml with a final concentration of 0.6% AcOH. The solution is then mixed (vortex) and ready for injection.

Compound (drug): Each compound is prepared and dissolved in the most suitable vehicle according to standard procedures.

#### (ii) Solutions administration

The compound (drug) is administered orally, intraperitoneally (i.p.), subcutaneously (s.c.) or intravenously (i.v.) at 10 ml/kg (considering the average mice body weight) 20, 30 or 40 minutes (according to the class of compound and its characteristics) prior to testing. When the compound is delivered centrally: Intraventricularly (i.c.v.) or intrathecally (i.t.) a volume of 5  $\mu\text{L}$  is administered.

The AcOH is administered intraperitoneally (i.p.) in two sites at 10 ml/kg (considering the average mice body weight) immediately prior to testing.

#### (iii) Testing



The animal (mouse) is observed for a period of 20 minutes and the number of occasions (Writhing reflex) noted and compiled at the end of the experiment. Mice are kept in individual "shoe box" cages with contact bedding. A total of 4 mice are usually observed at the same time: one control and three doses of drug.

5 For the anxiety and anxiety-like indications, efficacy has been established in the geller-seifter conflict test in the rat.

For the functional gastrointestinal disorder indication, efficacy can be established in the assay described by Coutinho SV *et al*, in American Journal of Physiology - Gastrointestinal & Liver Physiology. 282(2):G307-16, 2002 Feb, in the  
10 rat.

### **ADDITIONAL IN VIVO TESTING PROTOCOLS**

#### **Subjects and housing**

Naïve male Sprague Dawley rats (175-200g) are housed in groups of 5 in a temperature controlled room (22°C, 40-70% humidity, 12-h light/dark). Experiments  
15 are performed during the light phase of the cycle. Animals have food and water ad libitum and are sacrificed immediately after data acquisition.

#### **Sample**

Compound (Drug) testing includes groups of rats that do not receive any treatment and others that are treated with E. coli lipopolysaccharide(LPS). For the  
20 LPS-treated experiment, four groups are injected with LPS, one of the four groups is then vehicle-treated whilst the other three groups are injected with the drug and its vehicle. A second set of experiments are conducted involving five groups of rats; all of which receive no LPS treatment. The naïve group receives no compound (drug) or vehicle; the other four groups are treated with vehicle with or without drug. These are  
25 performed to determine anxiolytic or sedative effects of drugs which can contribute to a reduction in USV.

#### **Administration of LPS**

Rats are allowed to habituate in the experimental laboratory for 15-20 min prior to treatment. Inflammation is induced by administration of LPS (endotoxin of  
30 gram-negative E. coli bacteria serotype 0111:B4, Sigma). LPS (2.4µg) is injected

intracerebro-ventricularly (i.c.v.), in a volume of 10µl, using standard stereotaxic surgical techniques under isoflurane anaesthesia. The skin between the ears is pushed rostrally and a longitudinal incision of about 1cm is made to expose the skull surface. The puncture site is determined by the coordinates: 0.8 mm posterior to the bregma, 1.5 mm lateral (left) to the lambda (sagittal suture), and 5 mm below the surface of the skull (vertical) in the lateral ventricle. LPS is injected via a sterile stainless steel needle (26-G 3/8) of 5 mm long attached to a 100-µl Hamilton syringe by polyethylene tubing (PE20; 10-15 cm). A 4 mm stopper made from a cut needle (20-G) is placed over and secured to the 26-G needle by silicone glue to create the desired 5mm depth.

Following the injection of LPS, the needle remains in place for an additional 10 s to allow diffusion of the compound, then is removed. The incision is closed, and the rat is returned to its original cage and allowed to rest for a minimum of 3.5h prior to testing.

#### 15 **Experimental setup for air-puff stimulation**

The rats remains in the experimental laboratory following LPS injection and compound (drug) administration. At the time of testing all rats are removed and placed outside the laboratory. One rat at a time is brought into the testing laboratory and placed in a clear box (9 × 9 × 18 cm) which is then placed in a sound-attenuating ventilated cubicle measuring 62(w) × 35(d) × 46(h) cm (BRS/LVE, Div. Tech-Serv Inc). The delivery of air-puffs, through an air output nozzle of 0.32 cm, is controlled by a system (AirStim, San Diego Instruments) capable of delivering puffs of air of fixed duration (0.2 s) and fixed intensity with a frequency of 1 puff per 10s. A maximum of 10 puffs are administered, or until vocalisation starts, whichever comes first. The first air puff marks the start of recording.

#### **Experimental setup for and ultrasound recording**

The vocalisations are recorded for 10 minutes using microphones (G.R.A.S. sound and vibrations, Vedbaek, Denmark) placed inside each cubicle and controlled by LMS (LMS CADA-X 3.5B, Data Acquisition Monitor, Troy, Michigan) software. The frequencies between 0 and 32000Hz are recorded, saved and analysed by the

same software (LMS CADA-X 3.5B, Time Data Processing Monitor and UPA (User Programming and Analysis)).

### **Compounds (Drugs)**

5 All compounds (drugs) are pH-adjusted between 6.5 and 7.5 and administered at a volume of 4 ml/kg. Following compound (drug) administration, animals are returned to their original cages until time of testing.

### **Analysis**

The recording is run through a series of statistical and Fourier analyses to filter (between 20-24kHz) and to calculate the parameters of interest. The data are  
10 expressed as the mean  $\pm$  SEM. Statistical significance is assessed using T-test for comparison between naive and LPS-treated rats, and one way ANOVA followed by Dunnett's multiple comparison test (post-hoc) for drug effectiveness. A difference between groups is considered significant with a minimum p value of  $\leq 0.05$ . Experiments are repeated a minimum of two times.

15

### **EXAMPLES**

The invention will further be described in more detail by the following Examples which describe methods whereby compounds of the present invention may be prepared, purified, analyzed and biologically tested, and which are not to be  
20 construed as limiting the invention.

#### **INTERMEDIATE 1**

A mixture of 4-(bromomethyl)benzoic acid, methyl ester (11.2 g, 49 mmol) and trimethyl phosphite (25 mL) was refluxed under N<sub>2</sub> for 5 hrs. Excess trimethyl phosphite was removed by co-distillation with toluene to give INTERMEDIATE 1 in  
25 quantitative yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.20 (d, 2H, J=22 Hz, CH<sub>2</sub>), 3.68 (d, 3H 10.8 Hz, OCH<sub>3</sub>), 3.78 (d, 3H, 11.2 Hz, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 7.38 (m, 2H, Ar-H), 8.00 (d, 2H, J=8 Hz, Ar-H).

**INTERMEDIATE 2: 4-(4-Methoxycarbonyl-benzylidene)-piperidine-1-carboxylic acid *tert*-butyl ester**

To a solution of INTERMEDIATE 1 in dry THF (200 mL) was added dropwise lithium diisopropylamide (32.7 mL 1.5 M in hexanes, 49 mmol) at -78 °C.

5 The reaction mixture was then allowed to warm to room temperature prior to addition of *N-tert*-butoxycarbonyl-4-piperidone (9.76 g, 49 mmol in 100 mL dry THF). After 12 hrs, the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate (3 x 300 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to give a crude product, which was purified by flash chromatography to

10 provide INTERMEDIATE 2 as a white solid (5.64 g, 35%). IR (NaCl) 3424, 2974, 2855, 1718, 1688, 1606, 1427, 1362, 1276 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.44 (s, 9H), 2.31 (t, J=5.5 Hz, 2H), 2.42 (t, J=5.5 Hz, 2H), 3.37 (t, J=5.5 Hz, 2H), 3.48 (t, J=5.5 Hz, 2H), 3.87 (s, 3H, OCH<sub>3</sub>), 6.33 (s, 1H, CH), 7.20 (d J=6.7 Hz, 2H, Ar-H), 7.94 (d, J=6.7 Hz, 2H, Ar-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 28.3, 29.2, 36.19, 51.9, 123.7, 127.8,

15 128.7, 129.4, 140.5, 142.1, 154.6, 166.8.

**INTERMEDIATE 3: 4-Bromo-4-[bromo-(4-methoxycarbonyl-phenyl)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester**

To a mixture of INTERMEDIATE 2 (5.2 g, 16 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.0 g) in

20 dry dichloromethane (200 mL) was added a solution of bromine (2.9 g, 18 mmol) in 30 mL CH<sub>2</sub>Cl<sub>2</sub> at 0 °C. after 1.5 hrs at room temperature, the solution after filtration of K<sub>2</sub>CO<sub>3</sub> was condensed. The residue was then dissolved in ethyl acetate (200 mL), washed with water (200 mL), 0.5 M HCl (200 mL) and brine (200 mL), and dried over MgSO<sub>4</sub>. Removal of solvents provided a crude product, which was

25 recrystallized from methanol to give INTERMEDIATE 3 as a white solid (6.07 g, 78%). IR (NaCl) 3425, 2969, 1725, 1669, 1426, 1365, 1279, 1243 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.28 (s, 9H), 1.75 (m, 1H), 1.90 (m, 1H), 2.1 (m, 2H), 3.08 (br, 2H), 3.90 (s, 3H, OCH<sub>3</sub>), 4.08 (br, 3H), 7.57 (d, J=8.4 Hz, 2H, Ar-H) 7.98 (d, J=8.4 Hz, 2H, Ar-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 28.3, 36.6, 38.3, 40.3, 52.1, 63.2, 72.9, 129.0, 130.3, 130.4,

30 141.9, 154.4, 166.3.

**INTERMEDIATE 4: 4-[bromo-(4-carboxy-phenyl)-methylene]-piperidine-1-carboxylic acid tert-butyl ester**

A solution of INTERMEDIATE 3 (5.4 g 11 mmol) in methanol (300 mL) and 2.0 M NaOH (100 mL) was heated at 40 °C for 3 hrs. The solid was collected by  
5 filtration, and dried overnight under vacuum. The dry salt was dissolved in 40% acetonitrile/water, and was adjusted to pH 2 using concentrated HCl.

INTERMEDIATE 4 (3.8 g, 87%) was isolated as a white powder by filtration. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.45 (s, 9H, <sup>t</sup>Bu), 2.22 (dd, J=5.5 Hz, 6.1 Hz, 2H), 2.64 (dd, J=5.5 Hz, 6.1 Hz, 2H), 3.34 (dd, J=5.5 Hz, 6.1 Hz, 2H), 3.54 (dd, J=5.5 Hz, 6.1 Hz, 2H),  
10 7.35 (d, J=6.7 Hz, 2H, Ar-H), 8.08 (d, J=6.7 Hz, 2H, Ar-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 28.3, 31.5, 34.2, 44.0, 115.3, 128.7, 129.4, 130.2, 137.7, 145.2, 154.6, 170.3.

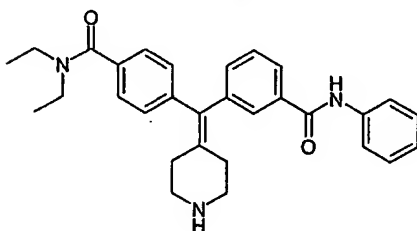
**INTERMEDIATE 5: 4-[bromo-(4-diethylcarbamoyl-phenyl)-methylene]-piperidine-1-carboxylic acid tert-butyl ester**

15 To a solution of INTERMEDIATE 4 (1.0 g, 2.5 mmol) in dry dichloromethane (10 mL) at -20 °C was added isobutylchloroformate (450 mg, 3.3 mmol). After 20 min at -20 °C diethylamine (4 mL) was added and the reaction was allowed to warm to room temperature. After 1.5 hrs the solvents were evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was  
20 washed with brine and dried over MgSO<sub>4</sub>. Removal of solvents provided a crude product, which was purified by flash chromatography to give INTERMEDIATE 5 as white needles (800 mg, 73%). IR (NaCl) 3051, 2975, 1694, 1633, 1416, 1281, 1168, 1115 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.13 (br, 3H, CH<sub>3</sub>), 1.22 (br, 3H, CH<sub>3</sub>), 1.44 (s, 9H, <sup>t</sup>Bu), 2.22 (t, J=5.5 Hz, 2H), 2.62 (t, J=5.5 Hz, 2H), 3.33 (m, 4H), 3.55 (m, 4H), 7.31  
25 (d, J=8.0 Hz, 2H, Ar-H), 7.36 (d, J=8.0 Hz, 2H, Ar-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 12.71, 14.13, 28.3, 31.5, 34.2, 39.1, 43.2, 79.7, 115.9, 126.3, 129.3, 136.8, 137.1, 140.6, 154.6, 170.5.

**INTERMEDIATE 6: 3-([1-(*tert*-butoxycarbonyl)piperidin-4-ylidene]{4-[(diethylamino)carbonyl]phenyl}methyl)benzoic acid**

To a flask containing INTERMEDIATE 5 (7.05 g, 15.6 mmol) was added toluene (150 mL), ethanol (150 mL), 3-carboxyphenylboronic acid (5.15 g, 31.0 mmol), and aqueous 2N sodium carbonate (46 mL, 92 mmol). The solution was degassed for 20 minutes, then palladium tetrakis(triphenylphosphine) (1.87 g, 1.62 mmol) was added. The reaction mixture was purged with nitrogen and heated to 90 °C. After 18 h, the reaction was cooled to rt and saturated aqueous ammonium chloride was added. The mixture was extracted with two portions of ethyl acetate and the combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting 50% to 100% ethyl acetate in hexanes, to yield INTERMEDIATE 6 as a pale yellow solid (5.76 g, 75%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 1.09-1.19 (m, 3H), 1.19-1.30 (m, 3H), 1.48 (s, 9H), 2.26-2.39 (m, 4H), 3.24-3.36 (m, 2H), 3.43-3.50 (m, 4H), 3.50-3.62 (m, 2H), 7.14 (d, J = 8.40 Hz, 2H), 7.19-7.24 (m, 1H), 7.33 (d, J = 8.20 Hz, 2H), 7.36-7.44 (m, 1H), 7.84-7.88 (m, 1H), 7.94-8.00 (m, 1H).

**COMPOUND 1: 4-[[3-(anilinocarbonyl)phenyl](piperidin-4-ylidene)methyl]-*N,N*-diethylbenzamide**



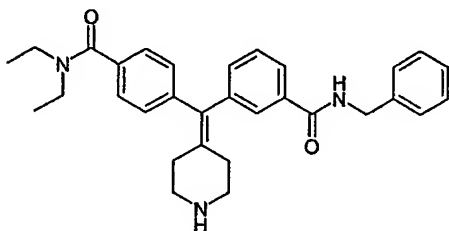
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To a solution of INTERMEDIATE 6 (0.110 g, 0.223 mmol) in dry DMF (5 mL) was added HATU (0.125 g, 0.329 mmol), DIPEA (100 µL, 0.574 mmol), and aniline (30 µL, 0.33 mmol). After 16 h the reaction mixture was concentrated. The residue was diluted with dichloromethane and washed with two portions of 2M NaOH, followed by brine. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was then dissolved in dichloromethane (10 mL) and 1 mL of trifluoroacetic acid was added. After 4 h, the reaction was concentrated and the residue was purified by reverse phase

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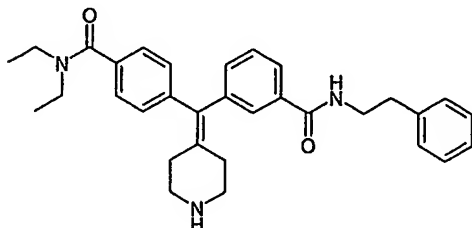
chromatography, eluting 10% to 45% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as the trifluoroacetic acid salt and was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to give COMPOUND 1 (80 mg, 60%) as a white solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.09 (t, J = 7.03 Hz, 3H), 1.20 (t, J = 7.03 Hz, 3H), 2.55-2.66 (m, 4H), 3.23-3.35 (m, 6H), 3.48-3.58 (m, 2H), 7.11-7.18 (m, 1H), 7.29-7.41 (m, 7H), 7.48 (t, J = 7.42 Hz, 1H), 7.63 (d, J = 7.62 Hz, 2H), 7.774-7.77 (m, 1H), 7.84-7.88 (m, 1H). Found: C, 61.35; H, 5.48; N, 6.47. C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>2</sub> x 1.50 CF<sub>3</sub>CO<sub>2</sub>H x 0.40 H<sub>2</sub>O has C, 61.37; H, 5.51; N, 6.51%.

10 **COMPOUND 2: 4-[[3-[(benzylamino)carbonyl]phenyl](piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



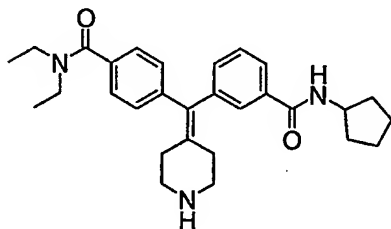
Using the same method as described for COMPOUND 1 and using INTERMEDIATE 6 (0.110 g, 0.223 mmol) and benzylamine (37 µL, 0.339 mmol) afforded COMPOUND 2 (80 mg, 60%) as its TFA salt. The product was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce a white solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.11 (t, J = 6.83 Hz, 3H), 1.23 (t, J = 6.83 Hz, 3H), 2.55 (q, J = 6.83 Hz, 2H), 2.61 (q, J = 6.83 Hz, 2H), 3.21-3.34 (m, 6H), 3.48-3.58 (m, 2H), 4.55 (s, 2H), 7.20-7.39 (m, 10 H), 7.46 (t, J = 7.62 Hz, 1H), 7.67-7.71 (m, 1H), 7.75-7.79 (m, 1H). Found: C, 61.14; H, 5.56; N, 6.26. C<sub>31</sub>H<sub>35</sub>N<sub>3</sub>O<sub>2</sub> x 1.60 CF<sub>3</sub>CO<sub>2</sub>H x 0.40 H<sub>2</sub>O has C, 61.19; H, 5.62; N, 6.26%.

**COMPOUND 3: 4-[(3-[(2-phenylethyl)amino]carbonyl)phenyl](piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



Using the same method as described for COMPOUND 1 and using INTERMEDIATE 6 (0.313 g, 0.635 mmol) and 2-phenylethylamine (0.12 mL, 0.95 mmol) afforded COMPOUND 3 (259 mg, 67%) as its TFA salt. The product was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce a white solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.12 (t, J = 7.23 Hz, 3H), 1.24 (t, J = 6.93 Hz, 3H), 2.53-2.58 (m, 2H), 2.58-2.63 (m, 2H), 2.86-2.93 (m, 2H), 3.22-3.34 (m, 6H), 3.49-3.61 (m, 4H), 7.15-7.30 (m, 7H), 7.31-7.35 (m, 1H), 7.37 (d, J = 8.40 Hz, 2H), 7.41-7.46 (m, 1H), 7.59-7.61 (m, 1H), 7.66 (ddd, J = 7.81, 1.76, 1.17 Hz, 1H). Found: C, 60.53; H, 5.81; N, 6.02. C<sub>32</sub>H<sub>37</sub>N<sub>3</sub>O<sub>2</sub> x 1.60 CF<sub>3</sub>CO<sub>2</sub>H x 1.10 H<sub>2</sub>O has C, 60.58; H, 5.89; N, 6.02%.

**COMPOUND 4: 4-[(3-[(cyclopentylamino)carbonyl]phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**

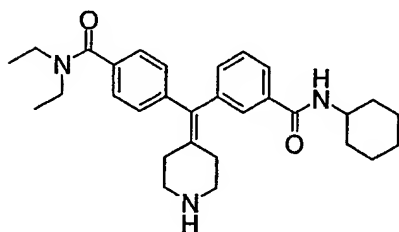


Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.300 g, 0.609 mmol) and cyclopentylamine (90 μL, 0.91 mmol). The crude material was purified by reverse phase chromatography, eluting 10% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce COMPOUND 4 (281 mg, 81%) as a white solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.12 (br t, J = 7.23 Hz, 3H), 1.23 (br t, J = 6.83 Hz, 3H), 1.51-1.69 (m, 4H), 1.72-1.81 (m, 2H), 1.96-2.06 (m, 2H), 2.53-2.58 (m, 2H), 2.58-2.63 (m,



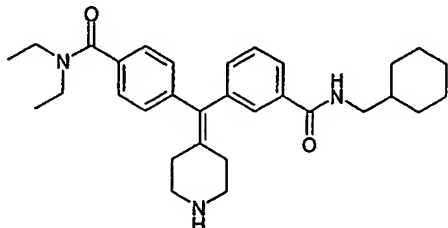
2H) 3.23-3.34 (m, 6 H) 3.50-3.57 (m, 2 H) 4.25-4.34 (m, 1 H) 7.28 (d,  $J = 8.40$  Hz, 2 H), 7.32 (ddd,  $J = 7.62, 1.76, 1.17$  Hz, 1H), 7.36 (d,  $J = 8.40$  Hz, 2H), 7.44 (t,  $J = 7.81$  Hz, 1H), 7.61-7.64 (m, 1H), 7.71 (ddd,  $J = 7.81, 1.76, 1.17$  Hz, 1H). Found: C, 58.86; H, 6.11; N, 6.19.  $C_{29}H_{37}N_3O_2 \times 1.7 CF_3CO_2H \times 0.4 H_2O$  has C, 58.90; H, 6.03; N, 6.36%.

**COMPOUND 5: 4-[[3-[(cyclohexylamino)carbonyl]phenyl](piperidin-4-ylidene)methyl]benzoic acid**



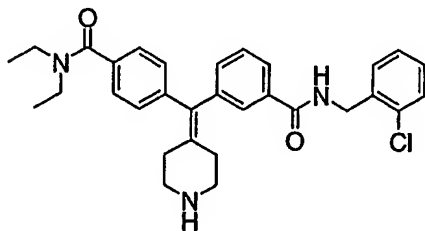
- 10 Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.300 g, 0.609 mmol) and cyclohexylamine (105  $\mu$ L, 0.91 mmol). The crude material was purified by reverse phase chromatography, eluting 10% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from  $CH_3CN/H_2O$  to produce
- 15 COMPOUND 5 (296 mg, 83%) as a white solid. Purity (HPLC): > 99%;  $^1H$  NMR (400 MHz,  $CD_3OD$ )  $\delta$  1.12 (br t,  $J = 6.44$  Hz, 3H), 1.17-1.27 (m, 4H), 1.28-1.46 (m, 4H), 1.64-1.72 (m, 1H), 1.77-1.84 (m, 2H), 1.89-1.96 (m, 2H), 2.53-2.58 (m, 2H), 2.58-2.63 (m, 2H), 3.22-3.34 (m, 6H), 3.49-3.58 (m, 2H), 3.79-3.89 (m, 1H), 7.28 (d,  $J = 8.40$  Hz, 2H), 7.33 (ddd,  $J = 7.62, 1.56, 1.17$  Hz, 1H), 7.36 (d,  $J = 8.20$  Hz, 2H), 7.44 (t,  $J = 7.42$  Hz, 1H), 7.62-7.64 (m, 1H), 7.71 (ddd,  $J = 7.81, 1.76, 1.17$  Hz, 1H).
- 20 Found: C, 60.75; H, 6.28; N, 6.45.  $C_{30}H_{39}N_3O_2 \times 1.6 CF_3CO_2H$  has C, 60.78; H, 6.24; N, 6.40%.

**COMPOUND 6: 4-[[3-(cyclohexylacetyl)phenyl](piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



- Synthesized by the same method as described for COMPOUND 1 using
- 5 INTERMEDIATE 6 (0.245g, 0.497 mmol) and cyclohexanemethylamine (97  $\mu$ L, 0.75 mmol). The crude material was purified by reverse phase chromatography, eluting 10% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to produce COMPOUND 6 (186 mg, 62%) as a white solid. Purity (HPLC): > 99%;  $^1\text{H}$
- 10 NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  0.92-1.04 (m, 2H), 1.12 (br t,  $J = 7.23$  Hz, 3H), 1.16-1.33 (m, 6H), 1.55-1.71 (m, 2H), 1.71-1.81 (m, 4H), 2.54-2.63 (m, 4H), 3.19 (d,  $J = 7.03$  Hz, 2H), 3.23-3.34 (m, 6H), 3.49-3.58 (m, 2H), 7.28 (d,  $J = 8.20$  Hz, 2H), 7.32-7.35 (m, 1H), 7.37 (d,  $J = 8.40$  Hz, 2H), 7.42-7.48 (m, 1H), 7.63-7.65 (m, 1H), 7.72 (ddd,  $J = 7.81, 1.17, 0.39$  Hz, 1H). Found: C, 61.44; H, 6.64; N, 6.31.  $\text{C}_{31}\text{H}_{41}\text{N}_3\text{O}_2 \times 1.4$
- 15  $\text{CF}_3\text{CO}_2\text{H} \times 0.7 \text{ H}_2\text{O}$  has C, 61.52; H, 6.69; N, 6.37%.

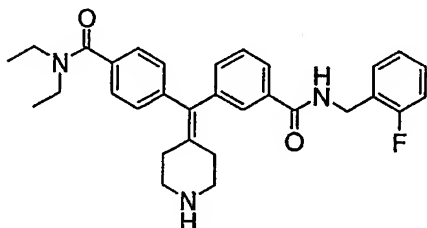
**COMPOUND 7: 4-[(3-[(2-chlorobenzyl)amino]carbonyl}phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



- 20 Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.337 g, 0.684 mmol) and 2-chlorobenzylamine (0.12 mL, 1.03 mmol). The crude material was purified by reverse phase chromatography, eluting 5% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to produce

COMPOUND 7 (280 mg, 65%) as a yellow solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.12 (br t, J = 6.64 Hz, 3H), 1.23 (t, J = 7.03 Hz, 3H), 2.53-2.65 (m, 4H), 3.21-3.35 (m, 6H), 3.48-3.59 (m, 2H), 4.62-4.67 (m, 2H), 7.23-7.31 (m, 4H), 7.34-7.42 (m, 5H), 7.45-7.50 (m, 1H), 7.70-7.71 (m, 1H), 7.80 (ddd, J = 7.81, 1.76, 1.17 Hz, 1H). Found: C, 58.57; H, 5.18; N, 6.09. C<sub>31</sub>H<sub>34</sub>N<sub>3</sub>O<sub>2</sub>Cl x 1.6 CF<sub>3</sub>CO<sub>2</sub>H x 0.2 H<sub>2</sub>O has C, 58.50; H, 5.17; N, 5.98%.

**COMPOUND 8: 4-[(3-[(2-fluorobenzyl)amino]carbonyl]phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



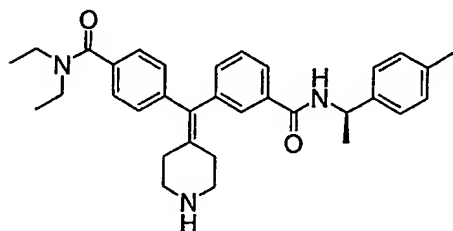
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Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.347 g, 0.704 mmol) and 2-fluorobenzylamine (0.12 mL, 1.06 mmol). The crude material was purified by reverse phase chromatography, eluting 5% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce COMPOUND 8 (132 mg, 31%) as a white solid. Purity (HPLC-215 nm): 97%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.12 (br t, J = 7.62 Hz, 3H), 1.23 (t, J = 6.83 Hz, 3H), 2.53-2.63 (m, 4H), 3.22-3.33 (m, 6H), 3.49-3.57 (m, 2H), 4.60-4.63 (m, 2H), 7.05-7.15 (m, 2H), 7.25-7.33 (m, 3H), 7.33-7.40 (m, 4H), 7.44-7.48 (m, 1H), 7.68-7.70 (m, 1H), 7.77 (ddd, J = 7.81, 1.76, 1.17 Hz, 1H). Found: C, 59.71; H, 5.30; N, 6.20. C<sub>31</sub>H<sub>34</sub>N<sub>3</sub>O<sub>2</sub>F x 1.6 CF<sub>3</sub>CO<sub>2</sub>H x 0.3 H<sub>2</sub>O has C, 59.75; H, 5.31; N, 6.11%.

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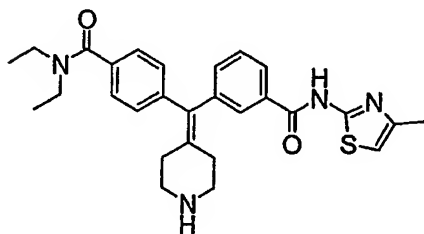
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**COMPOUND 9: 4-[[3-({[(1R)-1-(4-methylphenyl)ethyl]amino}carbonyl)phenyl](piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



- 5 Synthesized by the same method as described for COMPOUND 1 using  
INTERMEDIATE 6 (0.350 g, 0.710 mmol) and (R)-(+)- $\alpha$ -4-dimethylbenzylamine  
(0.16 mL, 1.07 mmol). The crude material was purified by reverse phase  
chromatography, eluting 5% to 50% acetonitrile in water containing 0.1%  
trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized  
10 from CH<sub>3</sub>CN/H<sub>2</sub>O to produce COMPOUND 9 (326 mg, 74%) as a grey solid. Purity  
(HPLC): > 99%; Optical Purity (Chiral HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  
 $\delta$  1.11 (br t, J = 6.64 Hz, 3H), 1.23 (br t, J = 6.25 Hz, 3H), 1.53 (d, J = 7.03 Hz, 3H),  
2.30 (s, 3H), 2.52-2.57 (m, 2H), 2.57-2.62 (m, 2H), 3.21-3.32 (m, 6H), 3.49-3.58 (m,  
2H), 5.29 (q, J = 7.03 Hz, 1H), 7.13 (d, J = 7.81 Hz, 2H), 7.23-7.30 (m, 4H), 7.31-  
15 7.34 (m, 1H), 7.36 (d, J = 8.40 Hz, 2H), 7.42-7.47 (m, 1H), 7.63-7.65 (m, 1H), 7.76  
(ddd, J = 7.81, 1.76, 1.17 Hz, 1H). Found: C, 61.05; H, 5.79; N, 5.75. C<sub>33</sub>H<sub>39</sub>N<sub>3</sub>O<sub>2</sub> x  
1.8 CF<sub>3</sub>CO<sub>2</sub>H x 0.3 H<sub>2</sub>O has C, 61.03; H, 5.79; N, 5.83%. [ $\alpha$ ]<sub>D</sub><sup>16</sup> = +10.8° (c 1.023,  
MeOH).

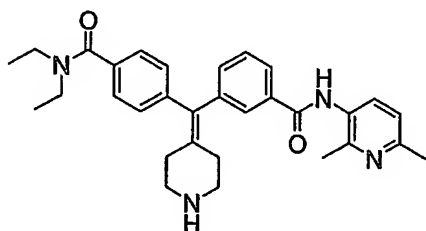
20 **COMPOUND 10: 4-[[3-({[(4-methyl-1,3-thiazol-2-yl)amino]carbonyl}phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



Synthesized by the same method as described for COMPOUND 1 using  
INTERMEDIATE 6 (0.305 g, 0.619 mmol) and 4-methyl-1,3-thiazol-2-amine (0.106

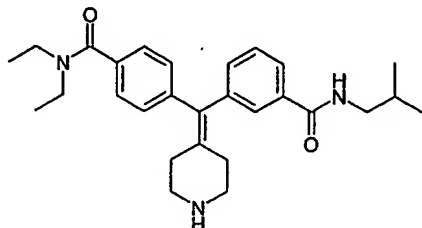
g, 0.929 mmol). The crude material was purified by reverse phase chromatography, eluting 5% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce COMPOUND 10 (270 mg, 72%) as a white solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.12 (br t, J = 6.64 Hz, 3H), 1.23 (br t, J = 7.03 Hz, 3H), 2.35 (d, J = 0.98 Hz, 3H), 2.57-2.65 (m, 4H), 3.25-3.34 (m, 6H), 3.49-3.57 (m, 2H), 6.74 (q, J = 0.98 Hz, 1H), 7.31 (d, J = 8.40 Hz, 2H), 7.38 (d, J = 8.40 Hz, 2H), 7.42-7.46 (m, 1H), 7.52-7.57 (m, 1H), 7.84-7.86 (m, 1H), 7.95 (ddd, J = 7.81, 1.76, 1.37 Hz, 1H). Found: C, 50.39; H, 4.56; N, 7.06. C<sub>28</sub>H<sub>32</sub>N<sub>4</sub>O<sub>2</sub>S x 2.4 CF<sub>3</sub>CO<sub>2</sub>H x 1.1 H<sub>2</sub>O has C, 50.37; H, 4.72; N, 7.16%.

**COMPOUND 11: 4-[(3-[(2,6-dimethylpyridin-3-yl)amino]carbonyl]phenyl)(piperidin-4-ylidene)-N,N-diethylbenzamide**



Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.315 g, 0.639 mmol) and 2,6-dimethylpyridin-3-amine (0.117 g, 0.340 mmol). The crude material was purified by reverse phase chromatography, eluting 5% to 45% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from CH<sub>3</sub>CN/H<sub>2</sub>O to produce COMPOUND 11 (350 mg, 90%) as a yellow solid. Purity (HPLC): > 99%; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.13 (br t, J = 6.83 Hz, 3H), 1.24 (t, J = 7.03 Hz, 3H), 2.57-2.65 (m, 4H), 2.67 (s, 3H), 2.76 (s, 3H), 3.24-3.35 (m, 6H), 3.49-3.58 (m, 2H), 7.31 (d, J = 8.59 Hz, 2H), 7.38 (d, J = 8.40 Hz, 2H), 7.48-7.52 (m, 1H), 7.55-7.60 (m, 1H), 7.75 (d, J = 8.40 Hz, 1H), 7.79-7.82 (m, 1H), 7.95 (ddd, J = 7.81, 1.95, 1.37 Hz, 1H), 8.43 (d, J = 8.40 Hz, 1H). Found: C, 51.81; H, 4.78; N, 6.60. C<sub>31</sub>H<sub>36</sub>N<sub>4</sub>O<sub>2</sub> x 3.0 CF<sub>3</sub>CO<sub>2</sub>H x 1.0 H<sub>2</sub>O has C, 51.87; H, 4.82; N, 6.54%.

**COMPOUND 12: 4-[(3-[(isobutylamino)carbonyl]phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



Synthesized by the same method as described for COMPOUND 1 using

- 5 INTERMEDIATE 6 (0.288 g, 0.585 mmol) and isobutylamine (87  $\mu$ L, 0.88 mmol).

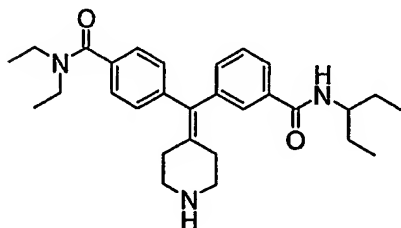
The crude material was purified by reverse phase chromatography, eluting 5% to 45% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to produce COMPOUND 12 (188 mg, 57%) as a white solid. Purity (HPLC): > 99%;  $^1\text{H}$  NMR (400 MHz,

- 10  $\text{CD}_3\text{OD}$ )  $\delta$  0.95 (d,  $J$  = 6.64 Hz, 6H), 1.12 (br t,  $J$  = 7.42 Hz, 3H), 1.22 (br t,  $J$  = 7.23 Hz, 3H), 1.07-1.16 (m, 1H), 2.53-2.64 (m, 4H), 3.17 (d,  $J$  = 7.03 Hz, 2H), 3.21-3.33 (m, 6H), 3.49-3.58 (m, 2H), 7.28 (d,  $J$  = 8.40 Hz, 2H), 7.32-7.39 (m, 3H), 7.45 (dd,  $J$  = 8.01, 7.62 Hz, 1H), 7.63-7.66 (m, 1H), 7.72 (ddd,  $J$  = 7.81, 1.76, 1.17 Hz, 1H).

Found: C, 60.22; H, 6.54; N, 7.19.  $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_2 \times 1.3 \text{ CF}_3\text{CO}_2\text{H} \times 0.8 \text{ H}_2\text{O}$  has C,

- 15 60.23; H, 6.59; N, 6.89%.

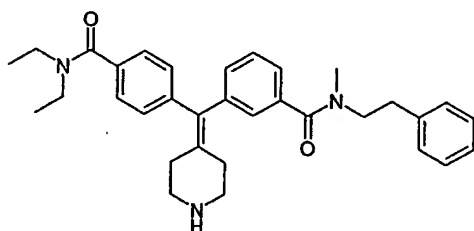
**COMPOUND 13: 4-[(3-[(1-ethylpropyl)amino]carbonyl]phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide**



- 20 Synthesized by the same method as described for COMPOUND 1 using INTERMEDIATE 6 (0.300 g, 0.609 mmol) and 1-ethylpropylamine (106  $\mu$ L, 0.913 mmol). The crude material was purified by reverse phase chromatography, eluting 10% to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. The product was obtained as its TFA salt and was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to produce

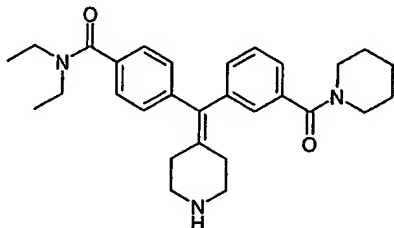
COMPOUND 13 (283 mg, 81%) as a white solid. Purity (HPLC): > 99%;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  0.93 (t,  $J = 7.42$  Hz, 6H), 1.12 (t,  $J = 6.64$  Hz, 3H), 1.24 (t,  $J = 6.64$  Hz, 3H), 1.46-1.69 (m, 4H), 2.54-2.64 (m, 4H), 3.23-3.33 (m, 6H), 3.50-3.58 (m, 2H), 3.83-3.93 (m, 1H), 7.29 (d,  $J = 8.40$  Hz, 2H), 7.32-7.39 (m, 3H), 7.45 (td,  $J = 7.62, 0.39$  Hz, 1H), 7.63-7.65 (m, 1H), 7.74 (ddd,  $J = 7.81, 1.76, 1.17$  Hz, 1H). Found: C, 59.29; H, 6.15; N, 6.61.  $\text{C}_{29}\text{H}_{39}\text{N}_3\text{O}_2 \times 1.7 \text{ CF}_3\text{CO}_2\text{H}$  has C, 59.37; H, 6.26; N, 6.41%.

COMPOUND 14: 4-[(3-{[methyl(2-phenylethyl)amino]carbonyl}phenyl)(piperidin-4-ylidene)methyl]-N,N-diethylbenzamide



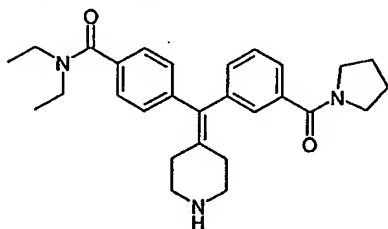
Using the same method as described for COMPOUND 1 and using INTERMEDIATE 6 (0.288 g, 0.585 mmol) and N-methylphenethylamine (0.13 mL, 0.89 mmol) afforded COMPOUND 14 (179 mg, 49%) as its TFA salt. The product was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to produce a white solid. Purity (HPLC): > 99%;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.02-1.17 (m, 3H), 1.18-1.28 (m, 3H), 2.49-2.64 (m, 4H), 2.72-2.80 (m, 2.5H), 2.97 (t,  $J = 7.23$  Hz, 1H), 3.11 (s, 1.5H), 3.18-3.33 (m, 6H), 3.45 (t,  $J = 7.23$  Hz, 1H), 3.48-3.58 (m, 2H), 3.76 (t,  $J = 7.42$  Hz, 1H), 6.82-6.85 (m, 1H), 6.88-6.94 (m, 1H), 6.99-7.06 (m, 1H), 7.15-7.30 (m, 7H), 7.31-7.45 (m, 3H). Found: C, 57.47; H, 5.36; N, 5.44.  $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_2 \times 2.4 \text{ CF}_3\text{CO}_2\text{H} \times 0.4 \text{ H}_2\text{O}$  has C, 57.43; H, 5.38; N, 5.32%.

**COMPOUND 15: N,N-diethyl-4-[[3-(piperidin-1-ylcarbonyl)phenyl](piperidin-4-ylidene)methyl]benzamide**



Using the same method as described for COMPOUND 1 and using INTERMEDIATE  
 5 6 (0.205 g, 0.416 mmol) and piperidine (62  $\mu$ L, 0.62 mmol) afforded COMPOUND  
 15 (185 mg, 77%) as its TFA salt. The product was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to  
 produce a white solid. Purity (HPLC): > 99%;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.12  
 (br t,  $J$  = 6.83 Hz, 3H), 1.23 (br t,  $J$  = 6.83 Hz, 3H), 1.42-1.50 (m, 2H), 1.59-1.73 (m,  
 4H), 2.56-2.62 (m, 4H), 3.23-3.33 (m, 8H), 3.49-3.57 (m, 2H), 3.64-3.71 (m, 2H),  
 10 7.17-7.19 (m, 1H), 7.26-7.33 (m, 4H), 7.37 (d,  $J$  = 8.40 Hz, 2H), 7.43-7.48 (m, 1H).  
 Found: C, 57.48; H, 5.79; N, 6.15.  $\text{C}_{29}\text{H}_{37}\text{N}_3\text{O}_2 \times 1.9 \text{ CF}_3\text{CO}_2\text{H} \times 0.5 \text{ H}_2\text{O}$  has C,  
 57.49; H, 5.87; N, 6.13%.

**COMPOUND 16: N,N-diethyl-4-{piperidin-4-ylidene[3-(pyrrolidin-1-ylcarbonyl)phenyl]methyl}benzamide**



Using the same method as described for COMPOUND 1 and using INTERMEDIATE  
 6 (0.212 g, 0.430 mmol) and pyrrolidine (54  $\mu$ L, 0.65 mmol) afforded COMPOUND  
 16 (189 mg, 78%) as its TFA salt. The product was lyophilized from  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  to  
 20 produce a white solid. Purity (HPLC): > 99%;  $^1\text{H}$  NMR (400MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.12  
 (br t,  $J$  = 7.42 Hz, 3H), 1.23 (br t,  $J$  = 6.64 Hz, 3H), 1.83-1.91 (m, 2H), 1.92-2.02 (m,  
 2H), 2.56-2.62 (m, 4H), 3.23-3.33 (m, 6H), 3.36-3.40 (m, 2H), 3.49-3.59 (m, 4H),  
 7.26-7.33 (m, 4H), 7.37 (d,  $J$  = 8.59 Hz, 2H), 7.43-7.47 (m, 2H). Found: C, 54.42; H,



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5.42; N, 5.89.  $C_{28}H_{35}N_3O_2 \times 2.3 CF_3CO_2H \times 0.6 H_2O$  has C, 54.48; H, 5.40; N, 5.85%.

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